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16. ABSTRACT

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In a second experiment, <24-h-old juveniles were fed *Artemia* nauplii from GSL, SPB and Brazil for 25 days. As in the first experiment, survival in the SPB treatment was lower than in the other two treatments. Growth was significantly greater in the GSL-fed mysids than in those fed the other two strains, which were similar. Reproductive potential was also found to be greatest in those mysids fed GSL *Artemia*.

These results are in marked contrast to those from a number of studies using other crustaceans which have found brine shrimp from either California or Brazil to be superior to those from GSL. Differences in the response to the various *Artemia* sources may be linked to the type of metamorphosis occurring during development. Those species which undergo a pronounced metamorphosis typically do not survive well on GSL *Artemia* while those in which metamorphosis is gradual or absent usually do.

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INTERNATIONAL STUDY ON *ARTEMIA*. XVI. SURVIVAL, GROWTH AND REPRODUCTIVE POTENTIAL OF THE MYSID, *MYSIDOPSIS BAHIA* Molenock FED VARIOUS GEOGRAPHICAL STRAINS OF THE BRINE SHRIMP, *ARTEMIA*[1]

D. MICHAEL JOHNS

United States Environmental Protection Agency, Environmental Research Laboratory, South Ferry Road, Narragansett, RI 02882, U.S.A.

WALTER J. BERRY

United States Environmental Protection Agency, Environmental Research Laboratory, South Ferry Road, Narragansett, RI 02882, U.S.A., and Graduate School of Oceanography, University of Rhode Island, Kingston, RI 02881, U.S.A.

and

WILLIAM WALTON

Department of Zoology, University of Maryland, College Park, MD 20742, U.S.A.

Abstract: Newly-hatched *Artemia* nauplii from different geographic locations were fed to *Mysidopsis bahia* Molenock to test their suitability as a food source for laboratory populations of the mysid. In one experiment, <24-h-old juveniles were fed *Artemia* for 10 days from one of the following locations: Macau, Brazil; Margherita di Savoia, Italy; San Pablo Bay (SPB), Calif., U.S.A.; Great Salt Lake (GSL), Utah, U.S.A.; Shark Bay, Australia. Survival over the 10-day period was significantly lower in the SPB strain than it was in the other strains. In addition, growth was greatest in the GSL and Brazil-fed juveniles, intermediate in those fed the Australian and Italian brine shrimp, and lowest for those fed the SPB strain.

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These results are in marked contrast to those from a number of studies using other crustaceans which have found brine shrimp from either California or Brazil to be superior to those from GSL. Differences in the response to the various *Artemia* sources may be linked to the type of metamorphosis occurring during development. Those species which undergo a pronounced metamorphosis typically do not survive well on GSL *Artemia* while those in which metamorphosis is gradual or absent usually do.

INTRODUCTION

A recent series of investigations has underscored the importance of the geographical origin of *Artemia* in determining their nutritional quality as a food source

[1] Contribution No. 186 of the Environmental Research Laboratory, Narragansett, RI 02882, U.S.A.

for marine species (Beck *et al.*, 1980; Johns *et al.*, 1980; Klein-MacPhee *et al.*, 1980). In these studies *Artemia* from five geographical locations were tested for their biological effectiveness in promoting good survival and growth in larvae of two species of crustaceans (*Rhithropanopeus harrisii* and *Cancer irroratus*) and two species of fish (*Pseudopleuronectes americanus* and *Menidia menidia*). The findings indicated that there are geographically-dependent differences in the nutritional quality of *Artemia* and that the response of test organisms to a brine shrimp diet is species-specific. Thus, the ability to predict the nutritional effectiveness of a particular geographical collection of *Artemia* for a specific organism is limited.

Differences in biological responses to the brine shrimp diet were found between those organisms which undergo a pronounced metamorphosis during development and those which do not. Larvae of the brachyuran crustaceans *Rhithropanopeus harrisii* and *Cancer irroratus* (Johns *et al.*, 1980) and of the winter flounder, *Pseudopleuronectes americanus* (Klein-MacPhee *et al.*, 1980) which were fed *Artemia* from Great Salt Lake, Utah, U.S.A. and San Pablo Bay, Calif., U.S.A. exhibited large increases in mortality at the time of metamorphosis. However, larvae feeding on brine shrimp collected from Margherita di Savoia, Italy, Shark Bay, Australia, and Macau, Brazil metamorphosed normally. Beck *et al.* (1980), on the other hand, found that the larvae of the Atlantic silverside, *Menidia menidia*, which do not undergo a pronounced metamorphosis, do not follow such a pattern of survival and growth when reared on these same five *Artemia* sources. Survival was greatest in those larvae fed the Brazil strain, intermediate in larvae fed the Italian and Australian strains and lowest in those fed the San Pablo Bay strain. However, *Menidia menidia* larvae fed the Great Salt Lake strain also exhibited intermediate survival rates.

The research presented in this paper was done to evaluate the nutritional quality of five geographical strains of *Artemia* for promoting survival, growth and reproductive capacity in the bay mysid, *Mysidopsis bahia* Molenock. Merhle *et al.* (1977) and the National Academy of Sciences (Anonymous, 1978b) have recently recognized the importance of nutritional factors in influencing the results of laboratory experiments. Subtle changes in physiological and metabolic parameters brought about by unrecognized nutritional variability may result in biased data and errors in interpretation. *M. bahia* has become a standard test organism in the U.S. Environmental Protection Agency Ocean Disposal Permit Program as well as other toxicological testing programs (Anonymous, 1978a). It is thus imperative that the responses of laboratory populations of *M. bahia* to different *Artemia* sources be determined in order to evaluate what biological differences may arise during continual maintenance of mysids on a brine shrimp diet.

Mysids are shrimp-like crustaceans which carry their developing embryos and larvae within a brood pouch. Young are released as juveniles, having metamorphosed from the larval to the juvenile life stage within the brood pouch. *Mysidopsis bahia* is a semitropical mysid (Molenock, 1969) that is a particularly easy organism

to maintain in the laboratory, lending itself to full life cycle toxicity tests (Bahner *et al.*, 1977; Nimmo *et al.*, 1977; Nimmo *et al.*, 1978).

MATERIALS AND METHODS

Geographical strains of *Artemia* tested for their nutritional quality were collected from the following areas: (1) Brazilian strain – from Macau, Brazil; (2) Italian strain – from Margherita di Savoia, Italy; (3) San Pablo Bay strain (SPB) – from San Pablo Bay, Calif., U.S.A.; (4) Great Salt Lake strain (GSL) – from the Great Salt Lake region, Utah, U.S.A.; (5) Australian strain – from Shark Bay, Australia. Further details about their origin and lot number can be found in Table I. To obtain

TABLE I

Summary of collection and incubation information on the five geographical strains of *Artemia* used in this study.

Artemia strain	Collection site	Incubation time (h)
Brazilian	Macau, Brazil (Companhia Industrial do Rio Grande do Norte, Cirne-Brand, harvested 1978)	24
Italian	Margherita di Savoia, Italy (harvested 1977)	29
Australian	Shark Bay, Australia (World Ocean, lot 113)	29
San Pablo Bay	San Pablo Bay Region, Calif., U.S.A. (Living World, lot 1628[a])	24
Utah	Great Salt Lake, Utah, U.S.A. (harvested 1977)	23

[a] Labelled as San Francisco Bay *Artemia* cysts, Living World, San Francisco Bay Brand, Inc. Apparently collected from San Pablo Bay instead of San Francisco Bay (P. Sorgeloos, pers. comm.).

stage I nauplii, cysts were incubated at 25 °C in 30‰ *S* filtered (0.45 μm) sea water for a specified period of time which was dependent on the particular geographical strain (Table I). Hatched nauplii were harvested at the end of this time period, carefully separated from hatching debris, and fed to the test organisms daily.

Prior to the initiation of the experiments, *Mysidopsis bahia* populations maintained in this laboratory were fed exclusively the Brazil strain for a period of 3 months. The decision to use this food source was based on the results of our previous work with crustaceans (Johns *et al.*, 1978, 1980) as well as the fact that this strain is very low in chlorinated hydrocarbon and heavy metal contamination (Olney *et al.*, 1980). The experiments were conducted in two stages, one in which the survival, growth and attainment of sexual maturity in juveniles were determined and one in which adult survival, growth, and brood size were monitored.

In the first experiment, newly-hatched juveniles (<24 h old) were placed in 10-cm finger bowls with five juveniles per bowl for a period of 10 days. The experiment was run three times, with three bowls per diet in each experimental run (total of 45 juveniles per diet). Rearing conditions for the juveniles were 25 °C – 30‰ *S*, a condition closely approximating that in the population tanks from which the juveniles were obtained. Daily, the juveniles were transferred to fresh sea water, examined for mortality, and then fed an excess of newly-hatched nauplii from the appropriate *Artemia* source. At the end of the 10-day period, the mysids were sexed and total length and dry weight measurements taken.

Total length measurements and sex determinations were made with a wide-field binocular microscope equipped with an ocular micrometer. Measurements were taken from the tip of the intraorbital processes to the tip of the uropod (excluding setae). Morphological characters which proved to be helpful in sexing 10-day-old mysids included the relative length of the pleopods, presence or absence of a brood pouch, and the presence of eggs in the oviduct. Following total length measurements, the mysids were rinsed in 0.9% ammonium formate (w:v), dried to a constant weight at 80 °C, and weighed to the nearest 1.0 μg on a Perkin-Elmer autobalance*. Dry-weight determinations were also made on a series of newly-hatched individuals.

In the second experiment, in which adult survival, growth, and brood size were estimated, 35 newly-hatched juveniles were placed in each of three 75-l aquaria. Only three *Artemia* strains could be tested in this experiment due to limitations of resources and space with the experiment repeated once. We chose to test the Brazil, GSL and SPB strains since the results of earlier work with crustacean larvae (Johns *et al.*, 1978, 1980) indicated that these three strains might provide us with a wide range of responses. The aquaria used in these experiments were modified for flow-through operation and equipped with undergravel filters. The substratum overlying the filter consisted of ≈ 15 mm of dolomite topped with 10 mm of beach sand. The water coming into the aquaria was pre-heated to 25 °C in a common head tank and had a salinity of between 29 and 32‰.

During the 25 days of the experiment, the mysids fed ad libitum on the appropriate food source which was replenished daily. At the end of the experimental period, all mysids were taken from each aquarium, counted, sexed, and measured. Methods for sexing and measuring these mysids were similar to those described in the previous experiment. In addition to these data, the number of embryos in the brood pouch of the gravid females was also determined.

One-way analysis of variance was computed on the survival data from both experiments. In order to normalize survival data, survival rates were transformed to angles (arcsine $\sqrt{\text{proportion surviving}}$) prior to analysis (Snedecor & Cochran, 1967). Preliminary analyses of the total length and dry-weight data indicated no

* Use of trade names does not imply endorsement by the U.S. Environmental Protection Agency.

significant differences between males and females in each food source, therefore the data were combined. Data from each replicate were also combined since preliminary analysis revealed no significant differences between the replicates. One-way analysis of variance was computed for both the total lengths and dry weights of the mysids at the end of each experiment. If significant differences (at $P = 0.05$) were found between mysids fed the various food sources, a Duncan's Multiple Range test was used to determine where differences existed (Goodnight, 1979). In addition, linear regression analysis was used to determine the relationship of brood size to body length in gravid females. Analysis of covariance was employed to test for differences in the resulting linear regressions that were due to diet (Snedecor & Cochran, 1967).

RESULTS

These experiments establish that the survival, growth and reproductive potential of the mysid, *Mysidopsis bahia* is dependent on food source. Survival among juvenile mysids fed the SPB strain was significantly lower ($P \leq 0.05$) than for those juveniles fed any of the other *Artemia* strains tested (Fig. 1). No significant differences ($P > 0.05$) in survival, however, were found among juveniles fed the Brazilian, Italian, Australian, and GSL strains.

Mysids feeding on the GSL and Brazilian strains were significantly larger in terms of dry weight and total length ($P \leq 0.05$) than those fed the other brine shrimp

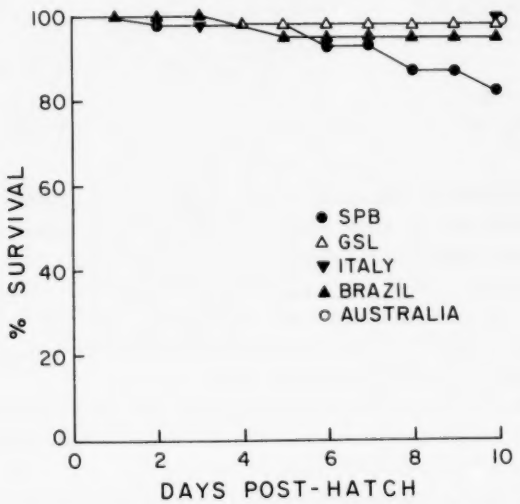

Fig. 1. Survival of juvenile *Mysidopsis bahia* fed five different geographical strains of *Artemia*.

sources (Tables II and III). SPB-fed mysids grew the least while those fed the Italian and Australian strains were of an intermediate weight. Taking the dry weight of a newly-hatched juvenile to be 22 ± 1.6 μg, there was an approximate 14.0-fold increase in the dry weight for mysids fed the GSL or Brazilian strain, but only an increase of 10.5 fold for mysids fed SPB during the same period of time.

TABLE II

Dry weight of *Mysidopsis bahia* juveniles cultured for 10 days on various geographical collections of *Artemia*: values are presented as a mean ± 1 SD; dry-weight values having the same grouping letter are not significantly different ($P \leq 0.05$); *n*, sample size.

Artemia strain	Dry wt (μg)	*n*	Grouping
Great Salt Lake	317 ± 48	37	A
Brazilian	299 ± 55	39	A
Italian	269 ± 46	39	B
Australian	264 ± 47	39	B
San Pablo Bay	231 ± 46	34	C

TABLE III

Total lengths for *Mysidopsis bahia* cultured on various geographical collections of *Artemia* for 10 days: values are presented as a mean ± 1 SD; total lengths having the same grouping letter are not significantly different ($P \leq 0.05$); *n*, sample size.

Artemia strain	Total length (mm)	*n*	Grouping
Great Salt Lake	5.08 ± 0.28	38	A
Brazilian	4.95 ± 0.28	42	A,B
Italian	4.87 ± 0.32	41	B,C
Australian	4.80 ± 0.25	39	C
San Pablo Bay	4.56 ± 0.36	34	D

Survival and growth of the newly-hatched juveniles placed in the 75-l aquaria for 25 days followed a pattern similar to that of the juveniles in the 10-day experiment. Approximately 80% of the mysids survived in the Brazilian- and GSL-fed tanks whereas only 43% survived in the SPB-fed tanks. However, over this period, mysids fed the GSL strain were significantly larger ($P \leq 0.05$) than those fed the Brazilian (Table IV). There were no significant differences ($P > 0.05$) in size between Brazilian-fed and SPB-fed adult mysids.

Various measures of reproductive potential were also found to differ with respect to nutritional source. In the 10-day experiment, ≈80% of the females offered the GSL strain were sexually mature (eggs in the oviduct), while only 25 to 30% of those mysids fed the Australian or SPB strains were sexually mature (Fig. 2).

Females fed the GSL brine shrimp were found to have a significantly greater

($P \leq 0.05$) number of embryos in the brood pouch (19 ± 5) than either Brazil- (10 ± 4) or SPB- (12 ± 7) fed females (Table V). In addition, linear regression analysis of the relationship between the number of embryos in the brood pouch and body size indicated that there were differences between the treatments.

TABLE IV

Total lengths for *Mysidopsis bahia* cultured on various geographical collections of *Artemia* for 25 days: values are presented as a mean $\pm$ 1 SD; total lengths having the same grouping letter are not significantly different ($P \leq 0.05$); *n*, sample size.

Artemia strain	Total length (mm)	*n*	Grouping
Great Salt Lake	7.42 ± 0.34	32	A
Brazilian	7.18 ± 0.18	60	B
San Pablo Bay	7.08 ± 0.31	63	B

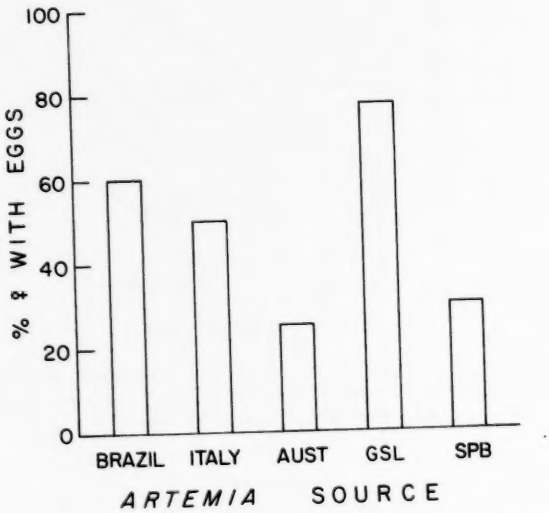

Fig. 2. Percentage of 10-day-old female *Mysidopsis bahia* having eggs in the oviduct after feeding on various geographical sources of *Artemia*.

Although the slopes of the fitted lines were not statistically different, the intercepts were, with the intercept for the GSL-fed group being significantly higher than for the other two groups (Fig. 3; Table VI). Therefore, the increase in brood size with increases in body size remained unaltered by food type, but the average number of embryos in the brood pouch for any given size of reproducing females did differ,

with individuals fed the GSL strain having a greater number of embryos in the brood pouch than those females offered the other two sources of brine shrimp.

Lastly, total juvenile production was affected by food source (Table V). At the

TABLE V

Brood size and the total number of juveniles produced in female *Mysidopsis bahia* fed various geographical collections of *Artemia*: values are presented as a mean ± 1 SD; *n*, sample size.

Artemia source	No. of eggs/brood pouch	*n*	Total no. of juveniles
Great Salt Lake	19 ± 5	25	79 ± 10
Brazil	10 ± 4	24	49 ± 7
San Pablo Bay	12 ± 7	7	32 ± 12

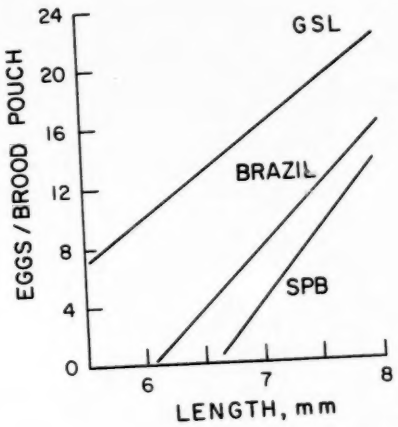

Fig. 3. Relationship of eggs in the brood pouch to total length in the mysid, *Mysidopsis bahia*, that had been fed on various geographical strains of *Artemia* for 25 days: pertinent statistics for the fitted regression lines can be found in Table VI.

TABLE VI

Fitted regression lines for the relationship of brood size to total length in the mysid *Mysidopsis bahia* fed various geographical collections of *Artemia*: those regression lines having the same grouping letter are not significantly different; *r*, correlation coefficient.

			Grouping	
Artemia strain	Regression line	*r*	Slope	Intercept
Utah	$Y = -17.8 + 4.98X$	0.46	A	A
Brazilian	$Y = -75.0 + 12.3X$	0.44	A	B
San Pablo Bay	$Y = -63.8 + 10.6X$	0.51	A	B

termination of the 25-day experiment, an average of 79 juveniles were found in the Utah-fed tanks while a mean of 49 and 32 juveniles were found in the Brazilian and SPB fed tanks, respectively.

DISCUSSION

The results presented in this study are considerably different from those reported for other crustaceans used to test the nutritional quality of geographical collections of *Artemia*. Forster & Wickins (1967), and Wickins (1972) reported that the larvae of the prawn, *Palaemon serratus*, did not survive well on a diet of GSL *Artemia*, but did when offered brine shrimp from San Francisco Bay. Subsequent research verified the relatively poor quality of nauplii from cysts collected from GSL compared to those from San Francisco Bay. Crustaceans tested included six species of brachyuran larvae (Reed, 1969; Bookhout & Costlow, 1970; Johns *et al.*, 1980) and the larvae of three species of shrimp (Forster & Wickins, 1967; Little, 1969; Reeve, 1969; Wickins, 1972; Provenzano & Goy, 1976).

Mysidopsis bahia, however, survived well when fed the GSL *Artemia*. In addition, growth rates of juvenile and adult stages as well as measurements of reproductive potential were greater in this study in those mysids fed the GSL strain. The value of 19 ± 5 embryos per brood pouch in Utah fed *M. bahia* is directly comparable to the value of 20 ± 3 which was obtained with *M. bahia* fed San Francisco Bay Brand *Artemia* (Nimmo *et al.*, 1980). Moreover, a review of the literature shows a dichotomy in the response of organisms to the GSL food source. Those species which undergo a radical metamorphosis during development (including crustaceans and some fish species) exhibit high mortality rates, with most of the mortalities occurring during metamorphosis (Little, 1969; Bookhout & Costlow, 1970; Johns *et al.*, 1980; Klein-MacPhee *et al.*, 1980). Species that do not have a pronounced metamorphic developmental pattern however, show no ill effects from a diet of GSL brine shrimp (Wickins, 1972; Beck *et al.*, 1980; this study).

The factors responsible for the variation in the nutritional quality of the five geographical strains used in this study are unknown despite extensive biochemical analysis. Seidel *et al.* (1980) reported similarities in amino acid compositions and protein content while Soejima *et al.* (1980) found little difference in the cartenoid compositions. Schauer *et al.* (1980), on the other hand, established differences in their fatty acid profile which might account for the differences in biological effectiveness.

Any cause–effect relationship is further complicated, however, by the fact that these five collections of brine shrimp were contaminated to varying degrees with chlorinated hydrocarbons and heavy metals (Olney *et al.*, 1980). Bookhout & Costlow (1970) have suggested that differences in performance might be due to levels of pollutant contamination. They reported finding three times as much DDT

in the GSL strain when compared to the San Francisco Bay strain. However, Wickins (1972) did not find much difference in the DDT contamination levels between GSL and San Francisco Bay *Artemia*. Moreover, he reported that if GSL nauplii fed for at least 1 day on *Isochrysis*, the detrimental effects previously found with the GSL nauplii were alleviated.

It is apparent from the preceding studies that there are major biochemical and/or contaminant differences between geographical collections of *Artemia* and that these differences can result in variable responses from an organism feeding upon *Artemia*. In the present study, we have demonstrated both lethal and sublethal responses (reduced growth and reproductive potential) in *Mysidopsis bahia* during the entire life cycle. It would seem that on the basis of our results, SPB *Artemia* nauplii are an inferior food source for *Mysidopsis bahia*. However, it should be pointed out that, in the absence of comparative information, SPB *Artemia* would seem to be a good food source; the survival, growth and reproduction of SPB-fed mysids is low only in comparison to that in those mysids fed the other four strains. In most cases such comparative information is not available, and even less is known about the foods used to support animals in the laboratory than is known about our *Artemia*. In such cases the choice of food source may have far-reaching consequences, which might easily go undetected (Phillips & Buhler, 1979).

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